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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	SCH01.NP001
First Inventor or Application Identifier	SCHNEIDER, Jay S.
Title	TREATMENT OF PARKINSON'S DISEASE WITH OLIGONUCLEOTIDES
Express Mail Label No.	EE 468 270 877 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

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1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)*
(Submit an original and a duplicate for fee processing)
2. [X] Specification [Total Pages 21]
(preferred arrangement set forth below)
- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings *(if filed)*
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. [X] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 7]
4 Oath or Declaration [Total Pages 2]
a. [X] Newly executed (original or copy)
b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d))
(for continuation/divisional with Box 16 completed)
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Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F R. §§ 1.63(d)(2) and 1.33(b).

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5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission
(if applicable, all necessary)
a. [ ] Computer Readable Copy
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ACCOMPANYING APPLICATION PARTS

7. [X] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [X] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
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13. [X] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
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Docket Number (Optional): SCH01.NP001

Applicant, Patentee, or Identifier: SCHNEIDER, Jay S.
Application or Patent No.: Not known
Filed or Issued: November 5, 1999
Title: TREATMENT OF PARKINSON'S DISEASE WITH OLIGONUCLEOTIDES

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ATTORNEY DOCKET NO.: SCH01.NP001

TITLE: TREATMENT OF PARKINSON'S DISEASE WITH OLIGONUCLEOTIDES

INVENTOR: SCHNEIDER, Jay S.

"Express Mail" Label No. EE 468 270 877 US
Date of Deposit – November 5, 1999

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TREATMENT OF PARKINSON'S DISEASE WITH OLIGONUCLEOTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119 based upon U.S. Provisional Patent Application No. 60/107,191 filed November 5, 1998.

FIELD OF THE INVENTION

The present invention generally relates to the field of genetic therapy and a method of treatment of Parkinson's disease, and more particularly to the use of antisense oligonucleotides or triplex oligonucleotides introduced into specific brain structures to decrease the function of brain circuits known to be overactive in the Parkinsonian brain.

BACKGROUND OF THE INVENTION

Certain aberrations in the neurochemical circuitry of the brain's basal ganglia are known to result in Parkinson's disease. When dopamine neurons die (the primary pathology in Parkinson's disease), the loss of dopamine to the striatum (caudate nucleus and putamen) sets into motion a series of changes in the neural activity of other brain nuclei located downstream from the striatum. **Figure 1** shows the normal neurochemical ciricuitry of the brain's basal ganglia.

Current neurosurgical approaches to this problem include either destroying the internal globus pallidus (GPi) by pallidotomy or implanting electrodes for electrical stimulation of this region of the brain. There are major disadvantages to these current neurosurgical approaches. Pallidotomy is permanent; can have

serious side effects depending on the precision of the lesion; and can result in dementia and other problems due to destruction of fibers of passage going through the GPi en route to other structures. Electrical stimulation procedures require implantation of electrodes in the brain and a control box under the skin. Over time, a tissue reaction could occur, thereby rendering electrodes useless or dysfunctional. Moreover, chronic electrical stimulation can damage tissue, and epileptic foci kindling may develop as a result of chronic electrical stimulation.

Traditional pharmacotherapies also have serious drawbacks in that the neurochemicals that need to be down-regulated in target structures are ubiquitous in the brain. Systemic administration of inhibitors of these neurochemicals can cause seizures, psychosis, coma, and even death.

The current invention has a number of novel features which represent improvements over the current treatments for Parkinson's disease. In the present invention, molecular neurosurgery is utilized. This approach, through the use of specific antisense or triplex oligonucleotides obviates the problems presented by conventional neurosurgical and pharmacotherapeutic approaches. Via the molecular neurosurgery described more fully below, the current invention selectively alters the functional status of specific groups of neurons without interfering with the functioning of other nearby neurons. This selective approach is made possible by targeting the application of the oligonucleotides to specific neurochemicals in specific neural structures. More precisely, selective targeting occurs by introducing antisense or triplex expression vectors to provide more long-term changes in gene expression. In this way, the result is selective inhibition of the abnormal functioning of certain neural circuits in the Parkinsonian brain without interfering with the normal functioning of these neurochemicals in the rest of the brain. Additionally, by altering the concentration or sequence of the oligonucleotides, the invention makes it possible to titrate the degree to which abnormal activity is inhibited in the target circuits. This ability to titrate further ensures that the treatment only interferes with abnormal functions and does not cause undesirable side effects

The current invention uses a molecular biological method of treating Parkinson's disease. The antisense oligonucleotides of the present invention are short sequences of phosphorothioate nucleotides, designed in the laboratory. These oligonucleotides block the translation of messenger RNA (mRNA) into protein at the ribosome. (**Figure 2**).

Another way to prevent or decrease the expression of deleterious genes is to block transcription of DNA. This approach is also utilized in the present invention. Oligonucleotides, designed in the laboratory, form triplex structures and block the transcription site on target DNAs.

Antisense or triplex oligonucleotides are targeted to the internal globus pallidus and/or substantia nigra pars reticulata (SNr) where the expression of glutamic acid decarboxylase (GAD_{67}, GAD_{65}, or a combination of the two isoforms) is downregulated. Glutamic acid decarboxylase (in the form of GAD_{67}, GAD_{65}, or a combination of the two isoforms) is the synthesizing enzyme for the production of the inhibitory neurotransmitter gamma aminobutyric acid (GABA). As a result of glutamic acid decarboxylase downregulation, a decrease occurs in the abnormally high output of GABA by the GPi and the SNr, and the symptoms of Parkinson's disease are ameliorated.

Other targets for the antisense or triplex oligonucleotide treatment of the present invention are the glutamate receptors on neurons in the GPi and the SNr. Decreased expression of these receptors blunts the response of these neurons to increased levels of glutamate released by projections from the subthalamic nucleus (STN) to the GPi and the SNr. (**Figure 3**). Another potential target for the antisense or triplex oligonucleotide treatment of the present invention are GABA receptors on neurons in the thalamic motor nuclei that receive excess inhibitory inputs from the GPi and the SNr.

Overall, the present invention is a treatment for Parkinson's disease, which is carried out by administering antisense oligonculeotides directed to the transcripts encoding the synthesizing enzyme GAD, thereby leading to a highly selective blockade of GABA. Although the actions of the antisense are selective,

they will be relatively short-lived. According to the literature, systems return to normal within a few days after cessation of treatment with antisense oligonculeotides. Typically, in order to demonstrate an effect on protein production, antisense oligonucleotides need to be administered repeatedly or by continuous infusion. As a result, the present invention employs RNA expression vectors. These eucaryotic expression vectors producing RNA antisense to GAD_{65} or GAD_{67} are injected into the desired brain region to transfect neurons so that when the cells receive a signal to produce GABA, the antisense would be activated and GABA is not produced.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata

for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is a combination of GAD_{67} and GAD_{65}.

It is another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of a triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of a triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of a triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is a combination of GAD_{67} and GAD_{65}.

It is an object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

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It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is a combination of GAD_{67} and GAD_{65}.

It is another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of a triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of a triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

It is a further object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of a triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase, wherein the isoform of said glutamic acid decarboxylase is a combination of GAD_{67} and GAD_{65}.

It is another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamate receptors.

It is another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamate receptors.

It is a another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the internal globus pallidus for the downregulation of glutamate receptors.

It is a another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamate receptors.

It is a another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the thalamic motor nuclei for the downregulation of GABA receptors.

It is a another object of the present invention to provide a method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the thalamic motor nuclei for the downregulation of GABA receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. A diagram of normal direct and indirect dopamine effects in the neurochemical circuitry of the brain's basal ganglia.

Figure 2. A schematic representation of how antisense oligonucelotide can block protein translation by hybridizing to the complementary glutamic acid decarboxylase mRNA. The antisense oligonucleotide hybridizes to the complementary target mRNA and causes a block of protein tranlation.

Figure 3. A diagram of the aberrations of the neurochemical circuitry of the brain's basal ganglia when dopamine loss occurs in the Parkinsonian brain.

Figure 4. A bar graph showing the percent reduction in apomorphine-induced rotations of GAD_{67} infused animals with experimentally-induced parkinsonism.

Figure 5-7. Bar graphs showing the relative changes in activity before and after treatment with GAD_{67} infused monkeys with experimentally-induced parkinsonism.

DETAILED DESCRIPTION

Oligodeoxynucleotides

Phosphorthioate oligonucleotides were synthesized by Biosynthesis Inc., Lewisville, TX. Since initial feasibility studies were conducted in rats, the first produced antisense oligonucleotides were directed against rat GAD sequences. The sequence used to generate rat GAD_{67} antisense was 5'TGGAAGATGCCATCAGCTCGG3' (SEQ ID NO:1). The sequence used to generate rat GAD_{65} antisense was 5'CCGGAGATGCCATGGGTTCTG3' (SEQ ID NO:2). The sequence used to generate human GAD_{65} is 5'CCGGAGATGCCATCGGCTTTG3' (SEQ ID NO:3). The sequence used to

generate human GAD_{67} is 5'TCGAAGACGCCATCAGCTCGG3' (SEQ ID NO:4). . The antisense sequence used for the monkey (*Saimiri sciureus*) studies was the GAD_{67}: 5'-GAAGATGGGGTCGAAGACGC-3' (SEQ ID NO:5). The control oligonucleotide for the monkey and rat GAD_{67} studies was a scrambled nucleotide sequence of the monkey GAD_{67} antisense sequence: 5'-TAGGAGCAGACTGAGAGGGCG-3' (SEQ ID NO:6).

These sequences were obtained by searching Genbank for the appropriate gene name. These sequences were analyzed using an open reading frame finder program at the National Center for Biotechnology Information World Wide Web site (http://www.ncbi.nlm.nih.gov/cgi-bin/gorf/orfig). The initiation of translation site was found and a 21 base antisense molecule complementary to the region spanning 8 bases 5' to 13 bases 3' (-8 to +13) to the initiation triplet was selected. These 21 base oligonucleotides were analyzed for cross reactivity with other genes using the NCBI BLAST server (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/).

The results of this search indicated that the oligos were only homologous with the genes they were directed against. Based on the BLAST algorithm, rat antisense molecules could react with GAD RNA of rat (*Rattus norvegicus*) and mouse (*mus musculus*). Further manual analysis of GAD_{67} revealed a 90.5% sequence identity between rat, pig (*Sus scrofa*) and human (*Homo sapien*) at the mRNA level surrounding the translation initiation site. Manual analysis of GAD_{65} sequences indicate 85.7% identify with human GAD_{65} in the region surrounding the initiation of translation site. Based on the BLAST algorithm, human GAD_{65} antisense molecules could react with human GAD-2 mRNA (a glutamate decarboxylase gene found in the human pancreas) and human GAD_{65}. The human GAD_{67} antisense molecule could react with human GAD_{67} (both in the brain and pancreas isoforms) and GAD_{67} from both pig and cat (*Felis cattus*).

The rat GAD_{67} antisense (SEQ ID NO:1) is composed of 23.8% adenosine, 23.8% cytosine, 33.3% guanine, and 19.0% thymine. The rat GAD_{65} antisense (SEQ ID NO:2) is composed of 14.3% adenosine, 23.8% cytosine, 38.1% guanine, and 23.8% thymine. The human GAD_{67} (SEQ ID NO:4)is composed of 23.8%

adenosine, 33.3% cytosine, 28.6% guanine, and 14.3% thymine. The human GAD_{65} (SEQ ID NO:3) is composed of 14.3% adenosine, 28.6% cytosine, 33.3% guanine, and 23.8% thymine. A missense molecule to be used in control experiments was designed by scrambling the base order of the antisense molecule. This control oligonucleotide for the GAD_{67} studies was a scrambled nucleotide sequence of the monkey (*Saimiri sciureus*) GAD_{67} antisense sequence: 5'-TAGGAGCAGACTGAGAGGGCG-3' (SEQ ID NO:6). The missense has the same percentages of each nucleotide as the antisense but the sequence has been changed. The missense oligonucleotides were analyzed using the NCBI BLAST server. The results of this analysis indicate that the missense molecules have no detectable homology with any known genes.

The antisense oligonucleotide was dissolved in sterile artificial cerebrospinal fluid (124mM NaCl, 1mM KCl, 2.4mM $CaCl_2$, 26mM $NaHCO_3$, 1.24mM NaH_2PO_4, 2mM D-glucose, and 1.3mM $MgSO_4$) to a final concentration of 43.1 μM. This resulted in an infusion of 21.5 μM/hr over a 2 week period.

Animals

Initial studies have been conducted in adult male Sprague-Dawley rats (226-250g). Animals were anesthetized with sodium pentobarbital and placed in standard stereotaxic frame. Animals were given unilateral lesions of the nigrostriatal dopamine system using the neurotoxin 6-hydroxdopamine-hydrobromide (6-OHDA-HBr). Two injections of 8mM 6-OHDA-HBr in 0.9% NaCl containing 1.4 mM ascorbic acid were made into the substantial nigra pars compacta (SNc) on one side of the brain. One injection was placed into the medial SNc and the other into the lateral SNc. Four weeks after lesioning, rats were assessed for the degree of rotational asymmetry after injection with the dopamine agonist apomorphine. Apomorphine-induced rotations in a direction away from the side of lesion is a standard measure of experimentally-induced parkinsonism in

rats. The ability of a drug to attenuate apomorphine-induced rotations is an accepted measure of anti-Parkinson efficacy. The number of complete 360 degree rotations was counted in 5-minute epochs over a total observation period of 1 hour. Rats rotating an average of 5 times per minute or more are considered to have at least 90% lesion to the nigrostriatal dopamine system. Animals with fewer rotations have lesser degrees of dopamine system damage.

Antisense Treatment

Once animals were shown to have lesion-induced rotations in response to apomorphine administration, they were randomly assigned to receive antisense treatment or sham infusions. The antisense was delivered via Alzet model 2002 mini asmotic pumps (Alza Corp. Palo Alto, CA), and a 32 gauge custom cannula (9.5mm below pedestal) which where made by Plastics One Inc (Roanoke, VA). These pumps are designed to pump at a rate of approximately 0.5μL/hr for 14 days. This results in a total antisense infusion of 7,241 pmols. The pumps and cannula were prepared according to manufacturer instructions. Briefly, the pumps were filled under sterile conditions with 250 microliters of either rat GAD_{67} antisense (SEQ ID NO:1)or control or missense (SEQ ID NO:6) vehicle solution. A flow moderator, cannula for brain infusion and 4mm of connector tubing were connected and attached to the pump. The pump assembly was then primed over night at 37°C in sterile saline. The pumps were implanted into animals the following day.

For pump implantation, rats were anesthetized with sodium pentobarbital and placed in a standard stereotaxic device on a homeothermic blanket. Core temperature was maintained at 37°C. In some animals the brain infusion cannula was implanted just above the entopeduncular nucleus (the rodent analog of the Gpi, 2.3mm behind bregma, 2.5mm lateral to the midline and 7.7 mm below the skull surface, according to the atlas of Paxinos and Watson). Other animals had

the brain infusion cannula implanted just above the substantia nigra pars reticulata (5.3mm behind bregma, 2.5mm lateral to the midline, 8.2mm below the skull surface, according to the atlas of Paxinos and Watson). These two targets were chosen because they represent the two major output areas of the basal ganglia. Overactivity of GABA-containing neurons in these brain regions are most likely responsible for the expression of parkinsonian symptoms. The cannulae were secured in place with dental acrylic. All animals had the Alzet pump placed in a subcutaneous pocket located between the scapulae on the back. Scalp wounds were closed with Autoclip wound clips and rats were kept warm until they regained consciousness.

Rats were again tested for apomorphine-induced rotations at 7 and 14 days after osmotic pump implantation. Fifteen to 17 days after pump implantation, animals were killed by decapitation and brains were immediately removed for analysis. Brains were removed and either flash frozen on dry ice for immunohistochemistry or microdissected for HPLC analysis of GABA content.

Additional antisense experiments were carried out whereby a single injection of antisense oligonucleotide was given to nine rats. These nine rats were lesioned with 6-OHDA, as described above. Approximately 3 to 4 weeks after lesioning, rats were tested for apomorphine-induced rotational asymmetry as described above. Once the integrity of the lesion was confirmed by a positive rotation response, animals were implanted with a guide cannula situated in the brain overlying the entopeduncular nucleus. Within weeks of recovery from surgery, rotational asymmetry was assessed again to re-establish a baseline response. Rats were then lightly anesthetized with an isofluorane/oxygen mixture and antisense oligonucleotide to GAD_{67} (SEQ ID NO:1)was slowly infused into the entopeduncular nucleus via an injection cannula inserted through the guide cannula. Each injection contained 250 ng of antisense in 0.5 µl.

Three squirrel monkeys (1 male, 2 female) were used for this pilot study. All animals had gross activity measures recorded while in an observation cage with use of an automated Doppler-based activity monitoring system. Activity

measures were first recorded when animals were normal. At least 3 to 5 activity sessions were recorded, each lasting at least 2 to 3 hours. Animals were then made parkinsonian by administration of several doses of the neurotoxin MPTP (1.5 to 2.5 mg/kg, i.m.). Toxin was administered until animals developed a stable parkinsonian syndrome. Animals were stably parkinsonian for a minimum of 6 months prior to antisense treatment. In preparation for antisense administration, animals were implanted with dual cannulae overlying the internal segment of the globus pallidus bilaterally. After surgery, activity measures were recorded once again to insure that there was no shift in baseline activity measures. After at least a week recovery period following surgery, the monkeys were restrained in a primate chair and antisense oligonucleotide was slowing infused into the internal globus pallidus sites via internal cannulae inserted through the implanted guide cannulae. For each experiment, GAD_{67} (SEQ ID NO:5) oligonulceotide was diluted fresh in sterile artificial cerebrospinal fluid (CSF). A total injection of 500ng was made, with 250ng injected into each of the sites on each side of the brain. The injection volume was 1.0 to 2.0 µl. Following administration of antisense, animals were returned to their cages and activity monitoring began 24 to 48 hrs. later and was recorded at various times over the next 2 to 3 wks. After antisense studies were performed, the same procedure was repeated using a missense oligonucleotide that was a scrambled sequence of the antisense oligonucleotide. Studies with the missense oligonucleotide (SEQ ID NO:5) were performed exactly as the antisense studies.

Results

In normal rats implanted with GAD_{67} antisense (SEQ ID NO:1) in the globus pallidus, HPLC analysis showed that a 2 week antisense infusion into the entopeduncular nucleus caused on average 65% reduction in GABA levels when compared with the contralateral untreated hemisphere.

Rats that had GAD_{67} antisense (SEQ ID NO:1) infused above the entopeduncular nucleus had the number of apomorphine-induced rotations reduced by an average of 52%. Rats that had the GAD_{67} antisense (SEQ ID NO:1) infused above the substantia nigra pars reticulata (SNr) had the number of apomorphine-induced rotations reduced by an average of 31%. The combined improvement in rotational asymmetry from both groups of animals is approximately 39%. Figure 4 is a graphical representation of these results. A change in the surgical coordinates may improve the response to SNr antisense infusion. Simultaneous infusions into both structures may also improve the response. In comparison, rats that received sham infusion showed an increase in apomorphine-induced rotations of 23% over the same time period.

Rats that had received the single injection method, apomorphine-induced rotations were reduced by an average of 34% when measured 24 hours after infusion. By 6 days after infusion, rotations were increased 5% above pre-antisense levels. The same rats received infusion of the scrambled oligonucleotide (SEQ ID NO:6) and these animals showed 25% and 38% increases in apomorphine-induced rotations 24 hours and 6 days, respectively, after control infusion.

These data demonstrate the feasibility and efficacy of this treatment as a means to reduce Parkinsonian symptomatology in a model commonly accepted for screening efficacy of potential new anti-Parkinson therapeutics.

The results of the monkey pilot study revealed that although the time courses and magnitudes of the effect differed slightly across the 3 animals, all animals showed an increase in spontaneous activity (a lessening of akinesia and bradykinesia) following antisense (SEQ ID NO:5) treatment but not following missense oligonucleotide (SEQ ID NO:6) treatment. Individual animal differences could have been due to slight differences in placement of the injection cannulae and individual differences in the degree of parkinsonism and levels of spontaneous activity. Nontheless, these non-human primate studies present proof of principle

that the therapy has potential beneficial effects on the major symptoms of Parkinson's disease.

CLAIMS

What is claimed is:

1. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase.

2. The method of claim 1 wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

3. The method of claim 1 wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

4. The method of claim 1 where in the isoform of said glutamic acid decarboxylase is a combination of GAD_{65} and GAD_{67}.

5. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamic acid decarboxylase.

6. The method of claim 5 wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

7. The method of claim 5 wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

8. The method of claim 5 wherein the isoform of said glutamic acid

decarboxylase is GAD_{65} and GAD_{67}.

9. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase.

10. The method of claim 9 wherein said isoform of said glutamic acid decarboxylase GAD_{65}.

11. The method of claim 9 wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

12. The method of claim 9 wherein the isoform of said glutamic acid decarboxylase is GAD_{65} and GAD_{67}.

13. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamic acid decarboxylase.

14. The method of claim 13 wherein the isoform of said glutamic acid decarboxylase is GAD_{65}.

15. The method of claim 13 wherein the isoform of said glutamic acid decarboxylase is GAD_{67}.

16. The method of claim 13 wherein the isoform of said glutamic acid decarboxylase is GAD_{65} and GAD_{67}.

17. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamate receptors.

18. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the substantia nigra pars reticulata for the downregulation of glutamate receptors.

19. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the internal globus pallidus for the downregulation of glutamate receptors.

20. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the internal globus pallidus for the downregulation of glutamate receptors.

21. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of antisense oligonucleotide to the thanlamic motor nuclei for the downregulation of GABA receptors.

22. A method of treating Parkinson's disease in a mammal, comprising administering a therapeutically effective amount of triplex oligonucleotide to the thanlamic motor nuclei for the downregulation of GABA receptors.

ABSTRACT

The present invention relates to a method of treatment of Parkinson's disease, and to the use of antisense oligonucleotides or triplex oligonucleotides introduced into targeted brain structures to decrease the function of brain circuits known to be overactive in the Parkinsonian brain. Antisense or triplex oligonucleotides are targeted to the internal globus pallidus and/or substantia nigra pars reticulata (SNr) where the expression of glutamic acid decarboxylase (GAD_{67}, GAD_{65}, or a combination of the two isoforms) is downregulated. The present invention also relates to a method of treatment of Parkinson's disease where antisense or triplex oligonucleotides are targeted to the internal globus pallidus and/or substantia nigra pars reticulata for the downregulation of glutamate receptors. The present invention further relates to a method of treatment of Parkinson's disease where antisense or triplex oligonucleotides are targeted to the thatlamic motor nuclei for the downregulation of GABA receptors.

09/352419 . 110599

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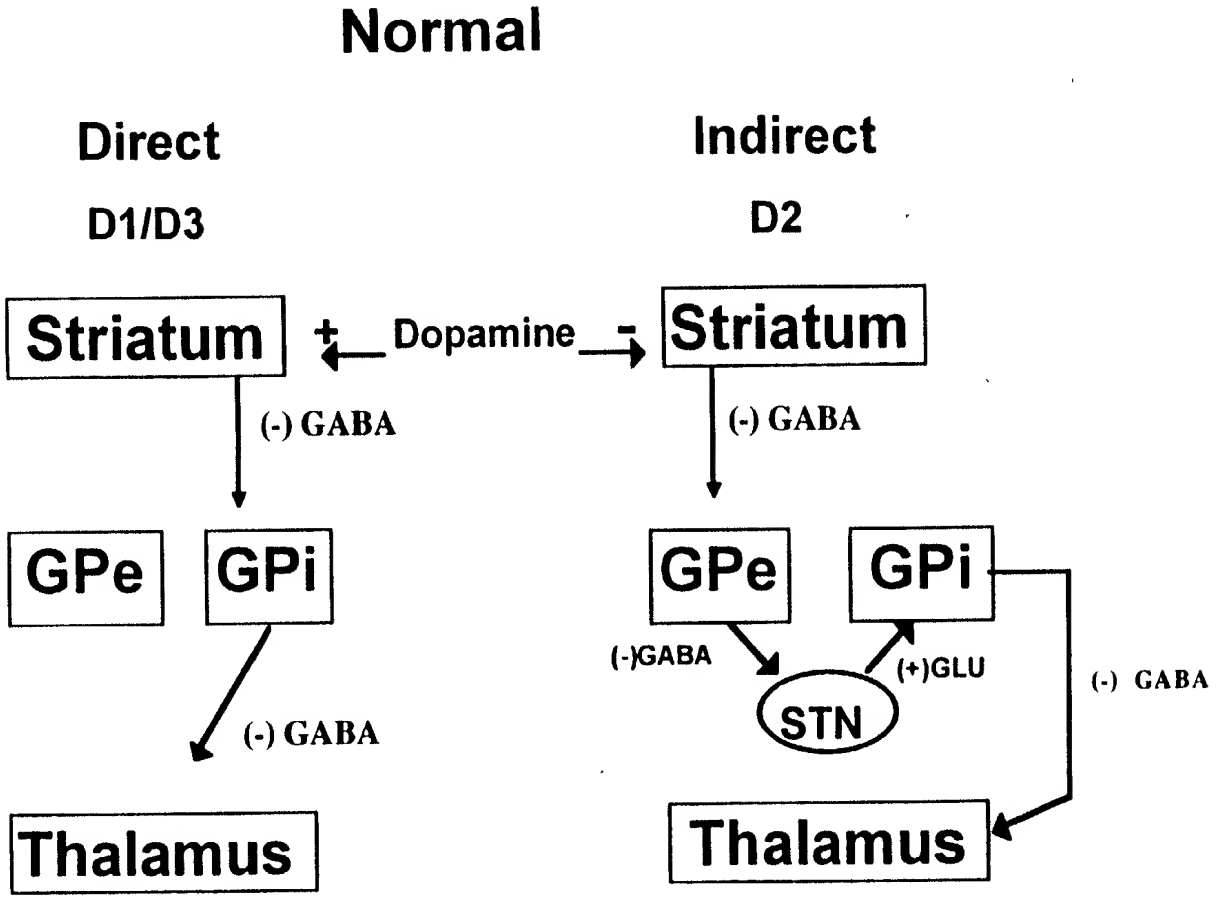

Fig. 1

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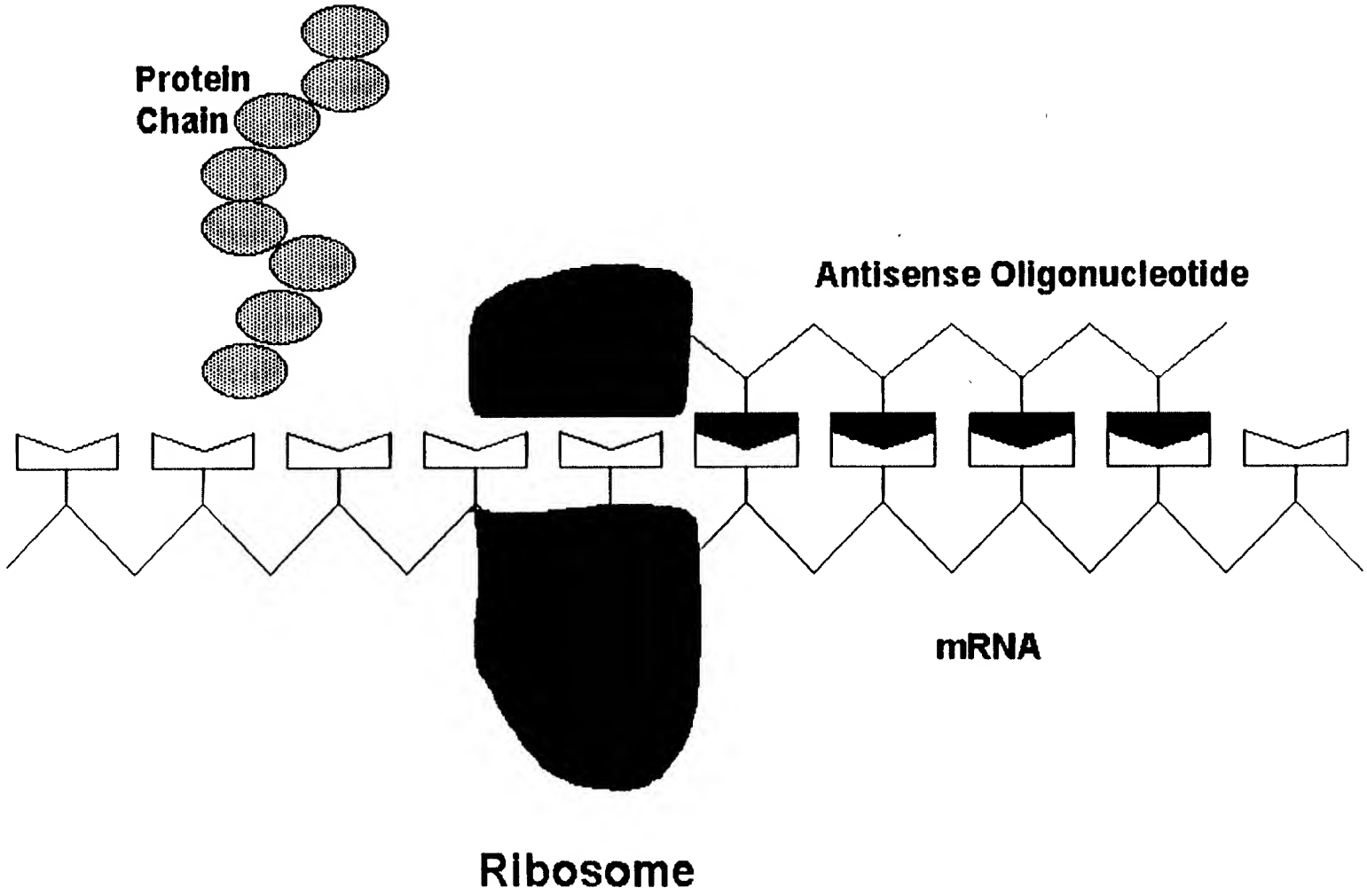


Fig. 2

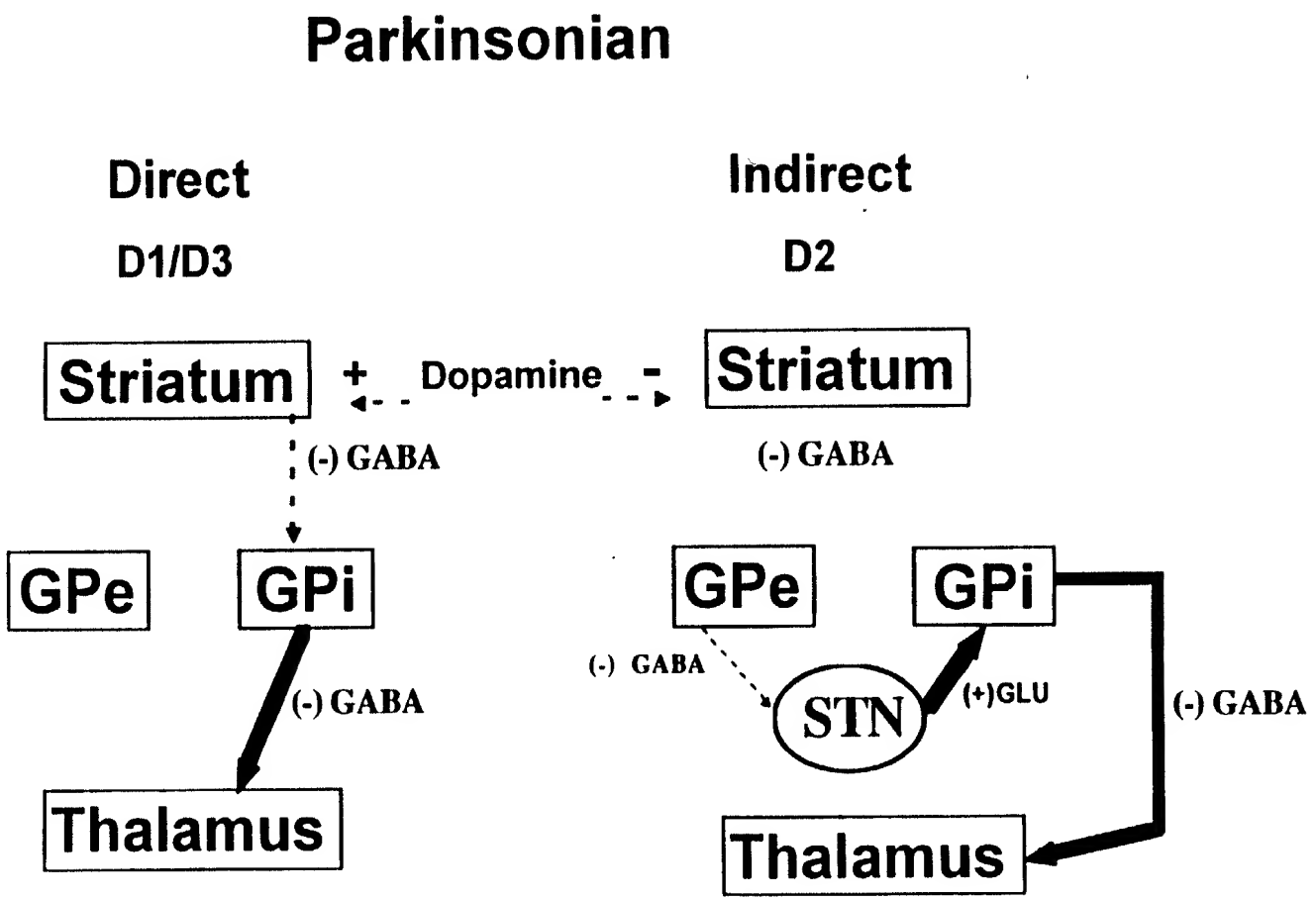
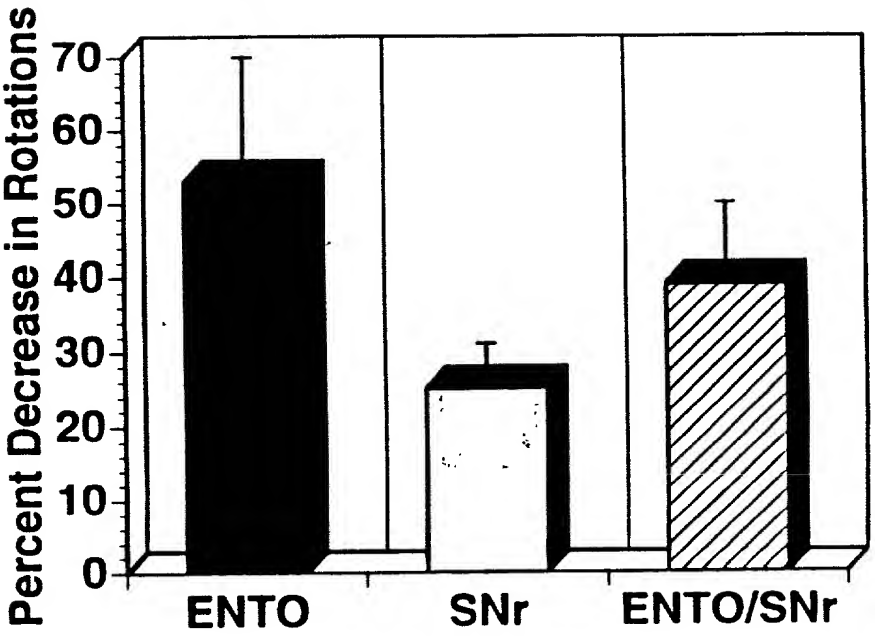

Parkinsonian
Direct
D1/D3
Indirect
D2
Striatum
+
Dopamine
-
Striatum
(-) GABA
(-) GABA
GPe
GPi
GPe
GPi
(-) GABA
(-) GABA
STN
(+)GLU
(-) GABA
Thalamus
Thalamus

Fig. 3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50
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20
10
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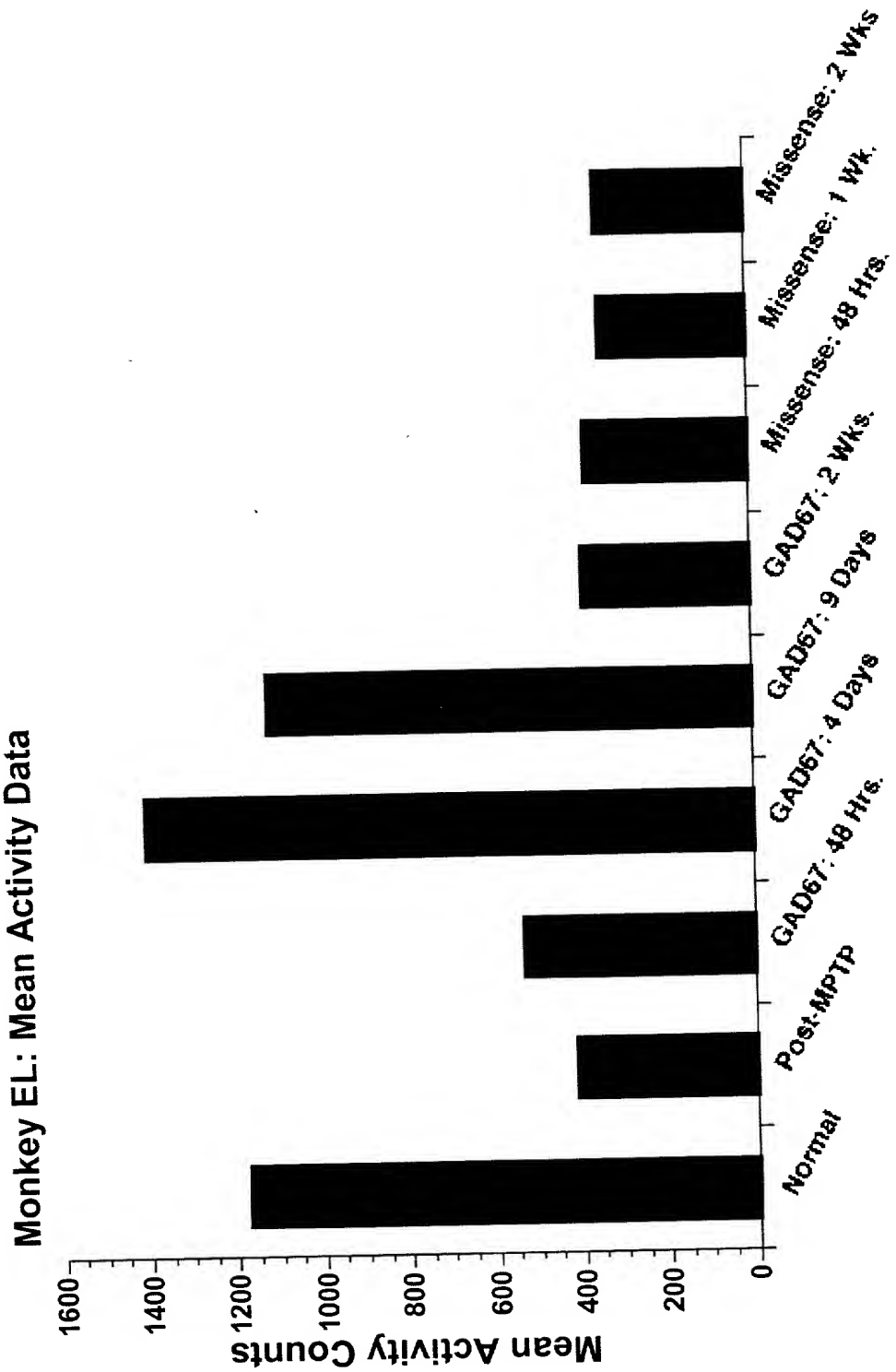
ENTO
SNr
ENTO/SNr

Fig. 4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Mean Activity Counts
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1200
1000
800
600
400
200
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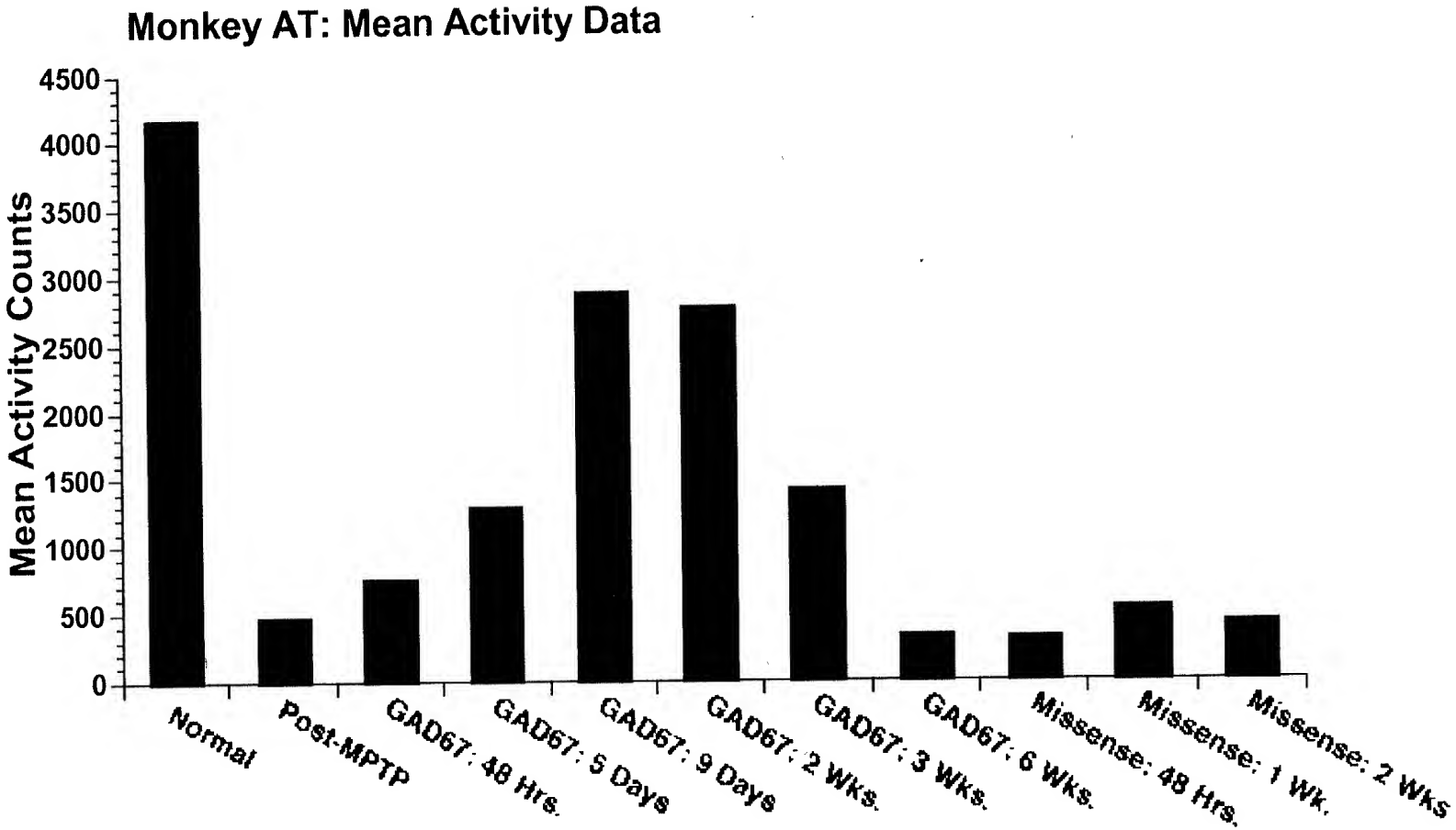
Post-MPTP
GAD67: 48 Hrs.
GAD67: 4 Days
GAD67: 9 Days
GAD67: 2 Wks.
Missense: 48 Hrs.
Missense: 1 Wk.
Missense: 2 Wks

Fig. 5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Mean Activity Counts
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4000
3500
3000
2500
2000
1500
1000
500
0
Normal
Post-MPTP
GAD67: 48 Hrs.
GAD67: 5 Days
GAD67: 9 Days
GAD67: 2 Wks.
GAD67: 3 Wks.
GAD67: 6 Wks.
Missense: 48 Hrs.
Missense: 1 Wk.
Missense: 2 Wks

Fig. 6

6650TT "6h25Eh60

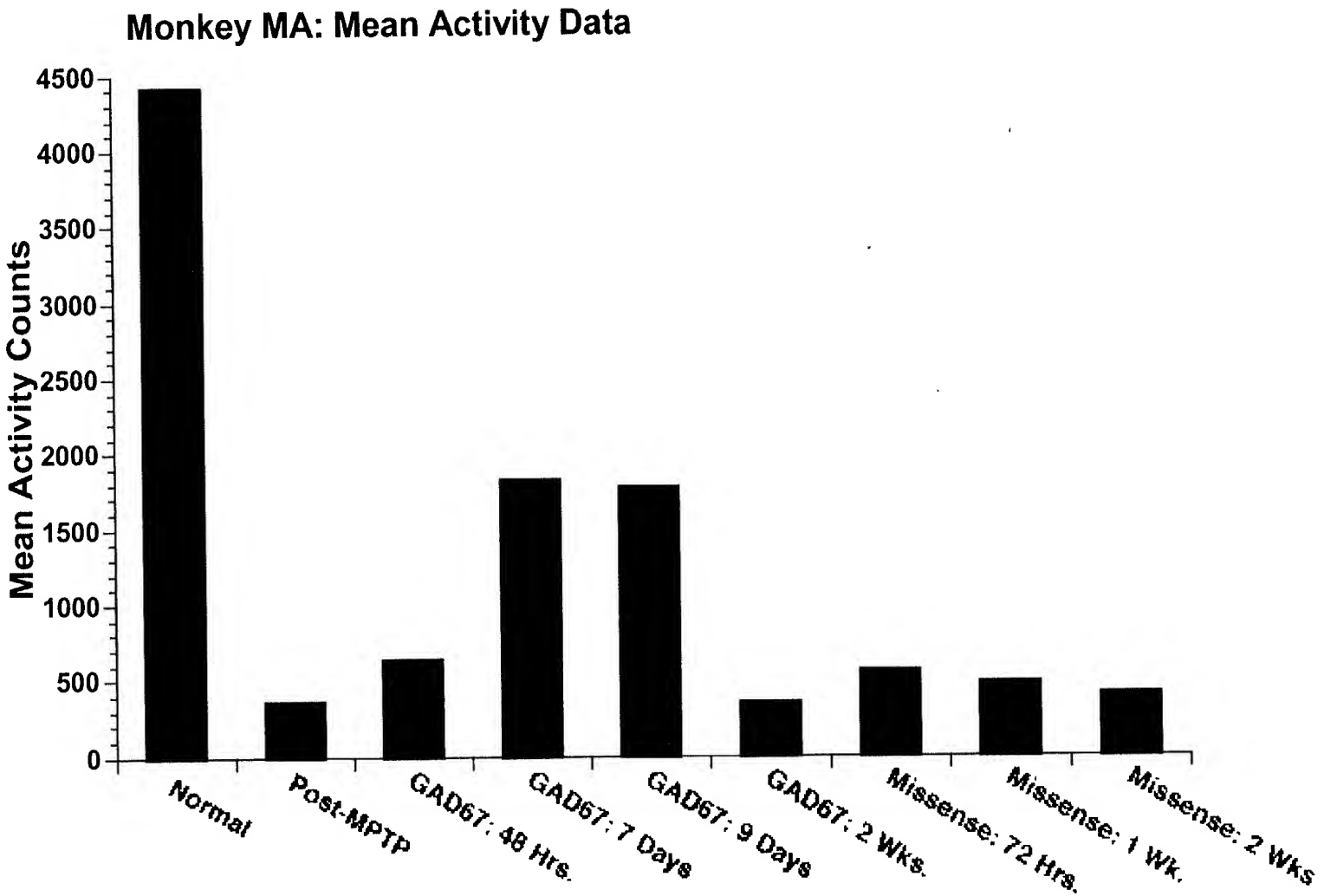

Fig. 7

SEQUENCE LISTING

<110> Schneider, Jay

<120> Treatment of Parkinson's Disease with Oligonucleotides

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PTO/SB/01 (12-97)
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Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☒ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	SCH01.NP001
First Named Inventor	SCHNEIDER, Jay S.
COMPLETE IF KNOWN	
Application Number	/
Filing Date	November 5, 1999
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TREATMENT OF PARKINSON'S DISEASE WITH OLIGONUCLEOTIDES

(Title of the Invention)

the specification of which
☒ is attached hereto
OR
☐ was filed on (MM/DD/YYYY) _____ as United States Application Number or PCT International Application Number _____ and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/107,191	11/05/1998	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*
OR
☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Clifford Kent Weber, Esq.	42,215		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] *OR* ☒ Correspondence address below

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City	Philadelphia	State	PA	ZIP	19107-5587
Country	USA	Telephone	(215) 503-0757	Fax	(215) 923-3613

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Jay S.	Schneider

Inventor's Signature	Jay Schneider					Date	11/05/99
Residence: City	Cherry Hill	State	NJ	Country	USA	Citizenship	USA
Post Office Address	1665 Blue Jay Lane						
Post Office Address							
City	Cherry Hill	State	NJ	ZIP	08003	Country	USA

☐ Additional inventors are being named on the ____supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto